PATENT

Docket No. 3922

Box Patent Application
Commissioner of Patents
and Trademarks
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s): Wilfred H. Nelson and Jay F. Sperry

WARNING: Patent must be applied for in the name(s) of all of the actual inventor(s). 37 CFR 1.41(a) and 1.53(b).

For (title): Direct Detection of Bacteria-Antibody Complexes Via UV Resonance Raman Spectra

1. Type of Application

This new application is for a(n) (check one applicable item below):

X Original

__ Design

__ Plant

NOTE: If one of the following 3 items apply then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

__ Divisional
__ Continuation
__ Continuation-in-part (CIP)

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date 03/14/97 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EM585001762US addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Tracy A. Sivacek
(Type or print name of person mailing paper)

Tracy A. Sivacek
(Signature of person mailing paper)

2. Benefit of Prior U.S. Application(s) (35 USC 120)

NOTE: If the new application being transmitted is a divisional continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

__ The new application being transmitted claims the benefit of prior U.S. application(s) and enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed Which Are Required For Filing Date Under 37 CFR 1.53(b) (Regular) or 37 CFR 1.153 (Design) Application

__8__ Pages of specification

__2__ Pages of claims

___ Pages of Abstract

__1__ Sheets of drawing

NOTE: "Identifying indicia such as the serial number, group and unit, title of the invention, attorney's docket number, inventor's name, number of sheets, etc., not to exceed 2 3/4 inches (7.0 cm) in width may be placed in a centered location between the side edges within three fourths inch (19.1 mm) of the top edge. Either this marking technique on the front of the drawing or the placement, although not preferred, of this information and the title of the invention on the back of the drawings is acceptable." Proposed 37 CFR 1.84(1). Notice of March 9, 1988 (1090 O.G. 57-62).

4. Additional papers enclosed

___ Preliminary Amendment

___ Information Disclosure Statement (37 CFR 1.98)

___ Form PTO-1449

___ Citations

___ Declaration of Biological Deposit

___ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

___ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

___ Special Comments

___ Other

5. Declaration or oath

__ Enclosed
executed by (check all applicable boxes)

__ inventor(s).

__ legal representative of inventor(s). 37 CFR 1.42 or 1.43

__ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

__ this is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. See Item 13 below for fee.

X Not Enclosed.

X Application is made by a person authorized under 37 CFR 1.41(c) on behalf of all the above named inventor(s). (The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

__ Showing that the filing is authorized. (Not required unless called into question. 37 CFR 1.41(d).

6. Inventorship Statement

WARNING: If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the **last** claimed invention was made, should be submitted.

The inventorship for all the claims in this application are:

X The same

or

__ Are not the same. An explanation, including the ownership of the various claims at the time the **last** claimed invention was made.

__ is submitted.
__ will be submitted.

7. Language

X English

__ non-English

__ the attached translation is a verified translation. 37 CFR 1.52(d).

8. Assignment

X An assignment of the invention to The Board of Governors for Higher Education, State of Rhode Island and Providence Plantations

__ is attached. A separate _____ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or __ FORM PTO 1595 is also attached.

X will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

9. Certified Copy

Certified copy(ies) of application(s)

(country)	(appln. no.)	(filed)
(country)	(appln. no.)	(filed)
(country)	(appln. no.)	(filed)

from which priority is claimed

__ is(are) attached.

__ will follow.

10. Fee Calculation (37 CFR 1.16)

A. X Regular application

CLAIMS AS FILED

Number filed	Number Extra		Rate	Basic Fee 37 CFR 1.16(a) $770.00
Total Claims (37 CFR 16(c)) 6 -20=	**0**	**X**	**$22.00**	$ 0.00
Independent Claims (37 CFR 1.16(b)) 2-3= 0		**X**	**$80.00**	$ 0.00
Multiple dependent claim(s), if any (37 CFR 1.16(d))			**$260.00**	$260.00

__ Amendment cancelling extra claims enclosed.
__ Amendment deleting multiple dependencies enclosed.
__ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $ 1,030.00

B. __ **Design application**
($300.00--37 CFR 1.16(f))
Filing Fee Calculation $_____

C. __ **Plant application**
($490.00--37 CFR 1.16(9))
Filing fee calculation $_____

11. Small Entity Statement(s)

__ Verified Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is(are) attached.

Filing Fee Calculation (50% of A, B or C above)
$_____

12. Request for International-Type Search (37 CFR 1.104(d)) (complete, if applicable)

__ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made At This Time

__ Not Enclosed

__ No filing fee is to be paid at this time. (This and the surcharge required by 37 CFR 1.16(e) can be paid subsequently.)

X Enclosed

X	basic filing fee	$ 1,030.00
__	recording assignment ($40.00; 37 CFR 1.21 (h))	$_____
__	petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached. ($130.00; 37 CFR 1.47 and 1.17(h))	$_____
__	for processing an application with a specification in a non-English language. ($130.00; 37 CFR 1.52(d) and 1.17(k)	$_____
__	processing and retention fee ($130.00; 37 CFR 1.53(d) and 1.21 (l))	$_____
__	fee for international-type search report ($35.00; 37 CFR 1.21 (e)).	$_____
	Total fees enclosed	$ 1,030.00

14. Method of Payment of Fees

X Check in the amount of $ 1,030.00

__ Charge Account No. _____ in the amount of $_____. A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: If no fees are to be paid on filing the following items should not be completed.
WARNING: Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.

X The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 19-0079 :

X 37 CFR 1.16(a), (f) or (g) (filing fees)

X 37 CFR 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

X 37 CFR 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

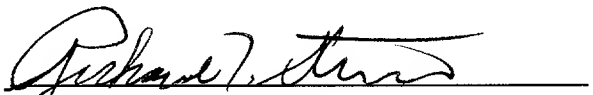
X 37 CFR 1.17 (application processing fees)

__ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311 (b))

16. Instructions As To Overpayment

X credit Account No. 19-0079

__ refund



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_ **Incorporation by reference of added pages**

Check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

_ Plus Added Pages For New Application Transmittal Where Benefit Of Prior U.S. Application(s) Claimed

Number of pages added __________

_ Plus Added Pages For Papers Referred To In Item 4 Above

Number of pages added __________

_ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added __________

X **Statement Where No Further Pages Added**

(If no further pages form a part of this Transmittal then end this Transmittal with this page and check the following item)

X This transmittal ends with this page.

Title

Direct Detection of Bacteria-Antibody Complexes Via UV Resonance Raman Spectroscopy

Field of the Invention

The detection and identification of microorganisms using Raman spectroscopy.

Background and Brief Summary of the Invention

There are many effective methods for the detection of microorganisms. At present, rapid, sensitive tests include fluorescent (fluorescence immunoassay or FIA), or radioactive labels (radio immunoassay or RIA) on the antibody attached in the antigen-antibody complex. Enzymes can be attached to the antibody to produce products which are more easily detected (ELISA). However, such processes (ELISA, RIA and FIA) tend to be labor intensive and not easily adapted to automation. The fluorescence method suffers from background interference and the RIA method is hampered by policies which discourage the use of radioisotopes in routine processes. If only small numbers of bacteria are present separation of the complex from the labelled antibody can be very difficult.

Among the most highly developed of the new rapid detection techniques is mass spectroscopy and its various combinations with gas chromatography (bacterial byproducts from cultures) and pyrolysis methods. Gas chromatography is highly effective in

detecting characteristic bacterial metabolic products. Flow cytometry provides for the rapid detection, identification, and separation of cells. Total luminescence spectroscopy can detect organisms very rapidly. The various immunological methods also can be very specific and very rapid. All of these methods have their distinct advantages and disadvantages.

Mass spectroscopy may be unequalled in identification of pure cultures and it is very rapid and sensitive. However, it is expensive to use, requires the destruction of samples, and is of questionable use in the analysis of complex mixtures. Flow cytometry is perhaps even more costly, requires extensive sample preparation, and in many aspects is limited in its scope of applicability. Luminescence techniques are of little use except in studies of pure cultures unless combined with immunological methods. Immunological methods are unequalled in specificity and speed, as well as sensitivity. Yet, they are often impractical to use unless very expensive and perishable materials are available in a state of constant readiness. Such methods are not practical for a wide range of organisms. Gas chromatography requires that cells be grown and, hence, this method is generally slow and of limited applicability.

In bacterial analysis normally the cost effective means of analysis involves isolating organisms and then growing them in controlled cultures. This process is very slow and relatively labor intensive.

It is known to detect and identify microorganisms based on

resonance Raman spectra, U.S. Pat. No. 4,847,198. A beam of visible or ultraviolet light energy contacts a microorganism under investigation. A portion of the light energy is absorbed by the microorganism and a portion of the light energy is 'emitted' from the microorganism at a lower energy level. The emitted light energy (resonance enhanced Raman scattering) can be correlated to a specific microorganism.

The present invention is directed to a system and method for detecting microorganisms with greater speed, sensitivity and specitivity than prior art methods. The need for growth of cultures is essentially eliminated. The sensitivity is much higher than rapid methods in current use (other than PCR and RIA) comparable to or better than RIA and better than FIA or ELISA since there is very little background interference and no need to purify or separate the complex.

The system and method of the invention avoids cumbersome separation steps and aids in the stabilization of the antigen-antibody complexes. This is especially true in those cases where it is necessary to detect small numbers of bacteria.

The invention is useful in environmental analysis for various consumer products, such as food products and liquid products and is useful for clinical analysis to provide rapid analysis of body fluids such as blood, spinal fluid or urine.

We have unexpectedly discovered that bacteria attached to antibody can be detected with resonance Raman spectroscopy. The bacteria can be detected directly in a great numerical excess, e.g.

100 to 10,000, of antibody molecules. This discovery results in a system and method for the rapid and low cost detection of microorganisms. The invention is based upon the formation and detection of the antigen-antibody complex. The detection of the complex is distinctly different from the prior art.

Broadly, the invention embodies a system and method for detecting microorganisms. A sample to be tested is placed in a medium, the medium containing antibodies attached to a surface for binding to a specific microorganism to form an antigen to antibody complex. The medium is contacted with a beam of light energy, some of the energy is emitted from the medium as a lower resonance enhanced Raman backscattered energy. The presence or absence of the microorganism is detected based on a characteristic spectral peak of said microorganism.

In the preferred embodiment, there is a rinse step before spectral analysis to isolate the antigen to antibody complex.

Brief Description of the Drawings

The figure illustrates a system of the invention.

Description of the Preferred Embodiment(s)

Example

Bacterium, *Escherichia coli*, was grown in 50 ml of Trypticase soy broth (without glucose) in a shaking waterbath at 37°C overnight. The bacteria were pelleted by centrifugation (12,100 x g for 5 minutes at 4°C), washed once in 20 ml of 0.85% saline and

resuspended in 5 ml of 0.025M sodium phosphate buffer pH 7 to which was added 2.25-25μl of anti-*Escherichia coli* (rabbit anti *E. coli* all ag's - purified IgG fraction 4-5 mg/ml protein, purchased from Biodesign International, Kennebunk, Maine). This was put into a continuous cycle loop, feeding through a quartz flow cell positioned in the laser beam.

Laser light 242 nm was directed into the flow cell. The emitted light energy (resonance enhanced Raman scattering) was sensed with a Raman detector. The spectrum was read and the prominent peak at 1485 cm^{-1} was easily detected. The tests confirmed that the spectral characteristics of the antibody are relatively weak and do not affect the spectra of the emitted light energy from the bacteria.

Detection of about 50 complexes in the presence of great excess (200-1000 fold) of antibody molecules was achieved. The number of complexes was estimated based on laser beam geometry and known bacterial densities in the culture studied.

The formation of a single wavelength in the ultraviolet range, the use of that wavelength to create spectral information about a specimen and the control and output of that information in various graphic or tabular forms is within the scope of those skilled in the art.

The figure illustrates a flow cell 10, a laser 12, a Raman detector 14 and a display 16.

If testing for salmonella in egg yolks, a sample of the egg yolk would be taken and placed in a fluid medium such as 0.025 M

phosphate buffer pH 7.2. Antibody, e.g. rabbit anti-salmonella antibodies attached to glass beads or another solid surface would then be mixed in the fluid medium. The medium would then be rinsed to remove other bacteria and contaminants and to isolate any bound antibody/salmonella complex. The isolate, preferably in aqueous medium, is placed in the cell 10. This isolate would then be scanned by the laser 12 as described above. The backscattered energy would be read by the detector 14. If the characteristic spectral peak of the bacterium were detected then the display 16 would indicate (actuate a light) the presence of salmonella in the source of the original sample.

Sensitive detection is possible because a prominent peak at 1485 cm^{-1} associated with nucleic acids of bacteria can be selectively and sensitively detected in the presence of proportionately very much larger numbers of antibody if irradiation is with laser light in the range 242-257.

Previous UV spectral studies of bacteria and protein support that, if the bacteria-antibody complex can be detected using 242 nm light, that the approach will work for various wavelengths in the vicinity of 242-257 nm for which there is little protein fluorescence interference in the Raman fingerprint region, and specifically at 1485 cm^{-1}.

The system and method also embodies microorganism/antibodies immobilized on various surfaces, i.e. magnetic beads, which allows for the application of simple "dip-stick" or immunomagnetic processes where antibody can be directly scanned by machine methods

for the presence of bacteria.

In an alternative embodiment, through use of inexpensive solar-blind coatings, analyses can be accomplished in full daylight. Since only a single peak, e.g. at 1485 cm^{-1}, needs to be detected, inexpensive detection methods normally used in UV filter fluorimetry can be used. This allows inexpensive optical components and simple detectors to be used.

The ability to sensitively detect bacteria in a great numerical excess of antibody results in an inexpensive means of scanning the surfaces containing immobilized antibody rapidly, sensitively and relatively inexpensively.

The suitable wavelength ranges for microorganisms and other cells are in the ultraviolet region (242-257 nm) which excites nucleotide bases of deoxyribonucleic and ribonucleic acids (DNA and RNA), as well as the aromatic amino acids of proteins (to a lesser extent).

Although the invention has been described with reference to the detection of a particular bacterium, it is equally applicable to the detection of any microorganisms or other cells that contain nucleic acids (DNA and/or RNA). Potentially, any cell that one can produce specific antibodies against for specific attachment could be detected using this detection system. In general, the common characteristics of the detection of the microorganisms is the presence of specific chemicals in their macromolecules, which when struck with an incident beam of light energy, particularly ultraviolet energy, emits very characteristic spectra.

The foregoing description has been limited to a specific embodiment of the invention. It will be apparent, however, that variations and modifications can be made to the invention, with the attainment of some or all of the advantages of the invention. Therefore, it is the object of the appended claims to cover all such variations and modifications as come within the true spirit and scope of the invention.

Having described our invention, what we now claim is:

1. A method for detecting microorganisms comprising:

placing a sample to be tested in a medium, the medium containing antibodies specific for binding to a microorganism to form an antigen to antibody complex;

contacting the medium with a beam of light energy, some of the energy emitted from the medium as a lower resonance enhanced Raman backscattered energy; and

detecting the presence or absence of the microorganism based on a characteristic spectral peak of said microorganism.

2. The method of claim 1 wherein the medium is a fluid medium and the microorganism is a bacterium.

3. The method of claim 2 wherein the light energy is ultraviolet light.

4. The method of claim 3 wherein the ultraviolet light is in the range of 242 to 257 nm.

5. The method of claims 3 or 4 wherein the medium is a liquid medium further comprising:

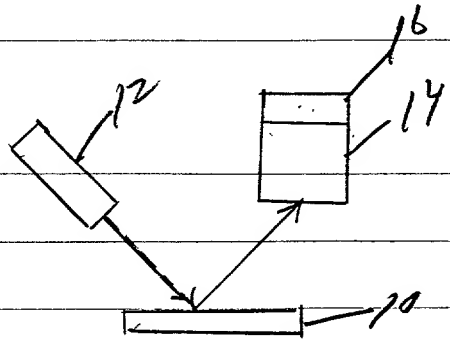
removing the antigen antibody complex from the liquid medium; and

detecting subsequently the presence or absence of the microorganism.

6. A system for detecting the presence or absence of a microorganism comprising:

contacting a medium containing antibodies specific for binding to a microorganism with a beam of light energy; and

means for detecting the presence or absence of the microorganism in the presence of an excess of antibodies.



Figure